REMARKS

Reconsideration of the allowability of the present application is requested respectfully.

Status of the Claims

Claims 1 to 11 were acted upon by the Examiner. No claims have been cancelled. Claim 1 has been amended. Claims 21 to 30 have been added. Support for Claims 21 to 30 may be found throughout the specification, specifically on page 2, line 28 to page 3, line 8, and page 23, lines 10 to 31. Accordingly, Claims 1 to 11 and 21 to 30 are presented for examination.

Summary of the Rejections

The Examiner has rejected Claims 1 to 5 and 7 to 10 under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 5,399,346 (Anderson et al. patent) taken with European Patent Application No. 0381490 (Greenberger et al. publication) and Boswell et al., Exp. Hematol. 11:315-323 (1983). The Examiner has rejected Claims 1 to 10 under 35 U.S.C. §103(a) as being unpatentable over the Anderson et al. patent, the Greenberger et al. publication, and the Boswell et al. publication, and in further view of Lozier et al., Hum. Gene Ther. 5:313-322 (1994). The Examiner has additionally rejected Claims 1 to 5 and 7 to 11 under 35 U.S.C. §103(a) as being unpatentable over the Anderson et al. patent, the Greenberger et al. publication, and the Boswell et al. publication, and further in view of Lobb et al. BBRC 178:1498-1504 (1991).

In addition, the Examiner has rejected Claims 1 to 11 under 35 U.S.C. §112, second paragraph, as being vague and indefinite.

Summary of the Present Invention

Transplantation of bone marrow stromal cells (BMSCs) is a promising therapy for diseases that involve hematopoietic cells. Given the effort required to isolate and transfect BMSCs and the potential therapeutic value of such cells, it is vital to preserve such transfected cells for later use. Furthermore, it is important that the nature of the process which is used to preserve the BMSCs is such that their properties are not affected adversely, for example, that their levels of expression of a transfected gene are not reduced or eliminated. The present invention is based on applicants' discovery that transfected BMSCs can be cryopreserved and thawed without a significant decrease in the expression level of the transfected gene.

Applicants' invention is defined in various claim forms. Method Claim 1 and the claims dependent thereon define the cryopreservation of transfected BMSCs which, upon being thawed, express a transfected exogenous gene at a level that is at least about 77% of the expression level of exogenous gene in transfected BMSCs prior to cryopreservation. Newly presented independent Claim 12 is directed to BMSCs which have been transfected with an exogenous gene and cryopreserved and which in the thawed state ("thawed BMSCs") express a transfected exogenous gene at a level that is at least about 77% of the expression level of the exogenous gene in transfected BMSCs prior to their being cryopreserved.

The 35 U.S.C. §103(a) Rejections

Applicants traverse respectfully the rejection of Claims 1 to 5 and 7 to 10 under 35 U.S.C. §103(a) as being unpatentable over the Anderson et al. patent taken with the Greenberger et al. publication and the Boswell et al. publication. The discussion which

follows will demonstrate that there is no logical basis to combine the disclosures of the cited publications and that each of the Examiner's rejections is based on hindsight.

The Anderson et al. patent discloses transfection of tumor-infiltrating lymphocytes (TILs) and the cryopreservation of transfected TILs. As acknowledged by the Examiner, this primary reference discloses neither BMSCs nor that the level of gene expression is relatively the same for transfected TILs and transfected, cryopreserved, and subsequently thawed TILs. Indeed, the primary reference contains a paucity of information concerning the cryopreservation of the TILs and no information whatsoever concerning the properties of the TILs in their thawed state. In this regard the Examiner's attention is directed respectfully to the passage cited by the Examiner (column 14 lines 37-38) which simply states "some of the TILs are then cryopreserved for further use in 10^{10} cell aliquots."

Notwithstanding the non-informative nature of the disclosure of the primary reference as regards applicants' claimed invention, the Examiner contends that, because the reference discloses the same steps as used in the process of the present invention, it would be expected that the level of expression of a transfected gene should be comparable to the level observed in the transfected cells of the present invention. There is no logical basis whatsoever for the Examiner to reach such a conclusion. The fact is that the cells of the present invention and those disclosed in the primary reference are significantly different. TILs are not BMSCs. TILs are a specific type of non-adherent, differentiated lymphocyte. In contrast, BMSCs are a heterogeneous population of adherent cells with varying levels of differentiation. Furthermore, the intracellular environment of a TIL contains a variety of expression regulatory elements different from those found in BMSCs. Accordingly, cryopreservation could have different effects on TILs relative to the effects of cryopreservation on BMSCs. It is abundantly clear that there is no sound reason to expect

that BMSCs have the same ability to express a transfected gene before and after cryopreservation as TILs--whatever that ability might be.

It is abundantly clear also that the secondary references do not in any way support the Examiner's obviousness rejections.

The Greenberger et al. publication discloses the use of BMSCs for gene therapy. It does not disclose cryopreservation of BMSCs and it does not disclose any information concerning TILs. The other "secondary reference" relied on by the Examiner is the Boswell et al. publication. It discloses cryopreservation of untransfected bone marrow cells. Furthermore, the Boswell et al. publication contains no information concerning TILs. Applicants are at a loss to understand the basis for combining the disclosures of the primary and secondary references inasmuch as the disclosures of the secondary references are not in any way related to the TILs disclosed in the primary reference.

It is submitted respectfully that the Examiner has relied on applicants' disclosure as a basis for combining the disclosures of the references. However, the basis to combine must be found in the publications themselves, not in the applicants' disclosure. It is clear that the Examiner's rejection is based on hindsight.

In view of the above, applicants respectfully request that the Examiner withdraw the rejection to Claims 1 to 5 and 7 to 10 under 35 U.S.C. §103(a) as being unpatentable over the Anderson et al. patent taken with the Greenberger et al. publication and the Boswell et al. publication.

As discussed below, the §103(a) rejections which additionally rely on the Lozier et al. publication and the Lobb et al. publication provide no basis to overcome the deficiencies of the Anderson et al. patent, the Greenberger et al. publication, and the Boswell et al. publication.

The §103(a) rejection of Claims 1 to 10 based on the disclosure of Anderson et al. patent, in view of the combined disclosures of the Greenberger et al. publication and the Boswell et al. publication, and in further view of the Lozier et al. publication, is traversed respectfully.

The Lozier et al. publication discloses transfected BMSCs, namely canine BMSCs transfected with Factor IX. Except for the use of canine BMSCs, the Lozier et al. publication does not disclose any relevant information beyond the information present in the Greenberger et al. publication. In fact, the Lozier et al. publication on page 320, first full paragraph, teaches a method which reverses the process steps of applicants' invention (and also the process steps disclosed in the Anderson et al. patent) suggesting that stromal cells could be frozen, thawed, and then transfected. Accordingly, applicants request respectfully withdrawal of the obviousness rejection of Claims 1 to 10 which relies on the Lozier et al. publication.

The Examiner's §103(a) rejection of Claims 1 to 5 and 7 to 11 as being unpatentable over the Anderson et al. patent, the Greenberger et al. publication and the Boswell et al. publication, and in further view of the Lobb et al. publication is traversed also.

The Lobb et al. publication discloses the expression of VCAM-1 in CHO cells followed by purification of the secreted recombinant protein.

The Lobb et al. publication provides no information regarding cryopreservation of transfected BMSCs. The Examiner has stated that the Lobb et al. publication discloses transducing B and T lymphocytes expressing VCAM-1. Applicants submit respectfully that the Examiner has mischaracterized the Lobb et al. publication. While the Lobb et al. publication does disclose transfection of CHO cells, it does not disclose transducing B and T lymphocytes with VCAM-1. However, even if Lobb did disclose transducing B and T

lymphocytes expressing VCAM-1, Lobb does not provide any information that overcomes the deficiencies in the other references.

Accordingly, applicants respectfully request withdrawal of the §103(a) rejection of Claims 1 to 5 and 7 to 11.

The 35 U.S.C. §112, Second Paragraph, Rejections

It is submitted that the amendments to Claim 1 overcome the Examiner's §112 rejection.

Applicants have amended Claim 1 to recite that the level of expression is "at least about 77%" of the level of expression of the exogenous gene in transfected BMSCs prior to their being cryopreserved. Support for this amendment can be found from page 19, line 26, to page 20, line 4. This section of the application indicates that thawed, cryopreserved, transfected BMSCs had an expression capacity of 445 μg of hGH/24 hour period as compared to an expression capacity of 575 μg of hGH/24 hour period for transfected BMSCs which have not been cryopreserved. As 445 divided by 575 equals 0.774, the expression capacity of the thawed, cryopreserved, transfected BMSCs is about 77% of the expression capacity of the transfected BMSCs which have not been cryopreserved. Given these results, applicants have amended Claim 1 to recite that the level of expression in the thawed, cryopreserved, transfected BMSCs is "at least about 77%" of the value possessed by non-cryopreserved, transfected BMSCs.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Enclosed in duplicate is an Amendment Transmittal which includes a Petition for extension of time to respond to the Examiner's Action and a check in payment of the extension and extra claim fees. The Commissioner is hereby authorized to charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 19-5425.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claim 1 has been amended as follows.

1. (Twice amended) A method for preserving[of obtaining a preparation of bone marrow stromal] cells [(BMSCs), the method]comprising:

(a) [transfecting cultured]providing bone marrow stromal cells (BMSCs) which have been transfected with an exogenous gene [to obtain](transfected BMSCs), the level of expression of the exogenous gene of the transfected BMSCs having a predetermined value; and

(b) cryopreserving the transfected BMSCs[, wherein the level of expression of the exogenous gene in the transfected and cryopreserved BMSCs is comparable to the] which in the thawed state have a level of expression of the exogenous gene [in transfected BMSCs that are not subsequently cryopreserved]which is at least about 77% of said predetermined value.

New Claims 21 to 30 have been added as follows.

--21. (New) Thawed BMSCs which have been transfected with an exogenous gene and cryopreserved, the level of expression of the exogenous gene of the thawed BMSCs being at least about 77% of the level of expression of said exogenous gene in the transfected BMSCs prior to cryopreservation.

22. (New) The BMSCs of Claim 21, wherein said BMSCs are human cells.

23. (New) The BMSCs of Claim 21, wherein said BMSCs are canine cells.

24. (New) The BMSCs of Claim 21, wherein said exogenous gene encodes a secreted peptide.

25. (New) The BMSCs of Claim 24, wherein the secreted peptide is a serum protein, a blood-clotting factor, a cytokine, a lymphokine, a growth factor, a peptide hormone, a lipid-binding protein, a metabolic enzyme, an antibacterial peptide, an antimicrobial peptide, an antifungal peptide, or a neurotransmitter.

26. (New) The BMSCs of Claim 25, wherein the blood-clotting factor is Factor VIII or Factor IX.

27. (New) The BMSCs of Claim 21, wherein the exogenous gene encodes a cell surface molecule.

28. (New) The BMSCs of Claim 27, wherein the cell surface molecule is V-CAM-1, I-CAM-1, N-CAM, or V-LAM.

29. (New) A method for preserving cells comprising:

(a) providing BMSCs

(b) transfecting said BMSCs with an exogenous gene (transfected BMSCs), the level of expression of the exogenous gene of the transfected BMSCs having a predetermined value; and

(c) cryopreserving the transfected BMSCs which in the thawed state have a level of expression of the exogenous gene which is at least about 77% of said predetermined value.

30. (New) The method of Claim 29, including thawing the cryopreserved transfected BMSCs.--